

# THE LABYRINTH

### **Indiana State Department of Health Laboratories Newsletter**

# Jamestown Canyon and Powassan — Two Arboviral Diseases of Growing Epidemiological Concern in the Northeastern and Midwestern United States (Part 1)

By Erica Vecchio, Microbiologist



Along with the advent of sunny skies, rising temperatures and outdoor recreation, summer brings the unfortunate increased risk of exposure to biting parasites such as flies, mosquitoes and ticks. Biologically classified as arthropods, these organisms may serve as vectors of disease transmission as well as inflicting pain and/or discomfort to their victims. More than 130 arthropod-borne viruses or arboviruses can occur when the virus is transmitted from an infected carrier to a host during blood-feeding on people (1-4). These pathogens may also be transmitted through blood transfusion, organ transplantation, perinatal transmission and breastfeeding or laboratory exposure. Human population expansion through urbanization, globalization and international travel, plus spiraling populations and climate change, have created conducive environments in which arthropods flourish.

Arboviruses have consequently emerged and expanded in the past 50 years, as rising threats to the human population (5,6). In 2017, 2,291 domestically-acquired cases of arboviral disease were reported by the CDC in the United States, 70% of which were neuroinvasive. Although 92% of these cases resulted from exposure to West Nile virus, increasing rates of infection of two relatively less known arboviruses, Jamestown Canyon virus (genus *Orthobunyavirus*) and Powassan virus (genus *Flavivirus*) in the northeastern and midwestern segments of the United States have created particular interest in health officials and medical personnel (3,5,7,8).

The genus *Orthobunyavirus* includes the California group of seroviruses, which are the only members of this genus found in North America. These viruses include La Crosse (LACV), California encephalitis (CE), Keystone, Snowshoe hare (SSHV), and Jamestown Canyon virus (JCV) (9,10). Although LACV encephalitis infection is generally better known than JCV infection, the number of cases of JCV exceeded the number of LACV in the United States in 2017 – 75 versus 63 (7,11). Additionally, the number of JCV infections appears to be rising yearly; 31 total cases were reported between 2000-2013, 11-15 cases annually in 2014-2016, and 75 total in 2017.

JCV, which was first isolated in 1961 from a *Culiseta inornata* mosquito pool in Jamestown Canyon, Colorado, was recognized as infectious to people in 1980 and became reportable to the Centers for Disease Control and Prevention (CDC) in 2004 (2, 11,3,20). Testing for JCV antibody began at the CDC in 2013, and the number of annual cases reported increased substantially (4, 12, 15). Questions have arisen as to whether the infection rate of the disease is truly increasing or whether improved testing methods and rising awareness of the disease have led to more cases; perhaps JCV disease has been underreported since its discovery as a human pathogen, due to the lack of diagnostic testing options.

Regardless of the cause, the CDC and other specialists in the field have recommended that JCV testing be considered during the diagnostic process when an arboviral agent is suspected in meningitis and/or encephalitis infections, when testing for more-recognized pathogens has yielded negative or inconclusive results, and when mosquito exposure is likely due to the season and/or geography of the case. Some health departments around the country, including the Indiana State Department of Health Laboratory (ISDHL), have either added or begun preparations to include JCV antibody testing through qualitative IgM capture ELISA within their serology departments.

(continued on next page)



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#### Our Mission:

The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for |surveillance and outbreak investigations to protect and improve Hoosier health.

#### **INDEX of Articles**

| Jamestown Canyon and Pow san Arboviruses         | as- |
|--|-----|
| Pages 1-3  |     |
| Indiana Non-Fatal Drug Abuse Surveillance Page 4 |     |

Detection of Sexually Transmitted Diseases

Page 5

Got Colistin?

\_\_\_\_\_Page 6-7

What's in your Water? Part 2

\_Page 8-11

Insuring Healthcare and Safety
One Swab at a Time

Page 12

Employee Spotlight

\_\_Page 13

## Jamestown Canyon and Powassan Arbovirus (continued from page 1)

The Wisconsin State Laboratory of Hygiene (WSLH), for example, has incorporated testing for both JCV and Powassan into its Arbovirus IgM Antibody Panel, that also includes testing for West Nile, La Crosse, Eastern equine encephalitis, and St. Louis encephalitis viral IgM antibodies (20).

In the majority of cases, human infections with arboviruses are asymptomatic or result in mild, flu-like illnesses. More specifically, exposure to Jamestown Canyon virus may be followed by no noticeable ailment or minimal to moderate sickness with conditions such as sore throat, fever, headache, muscle aches, fatigue, dizziness, rash, photophobia, nausea, and vomiting commonly appearing. In some patients, however, serious respiratory issues, confusion, seizures, encephalitis, meningitis, meningoencephalitis, and eventual death may occur. As of July 2019, at least three deaths from JCV have been reported to the CDC. Although the exact timeframe of symptom onset has not yet been confirmed, medical issues often appear in people between two days and two weeks after being bitten by an infected mosquito (15).

Additionally, victims appear to be predominately adult males with median age of 58 to 63 years, perhaps due to greater outdoor exposure and decreased likelihood of mosquito repellent use (8). Studies show that JCV is distributed widely throughout North America and resides primarily in white-tailed deer and several mosquito populations. Other wild mammals including bison, elk, moose, mule deer, pronghorn and a few domestic species, such as cattle, horses and sheep, may also harbor the pathogen. Humans and animals are classified as dead-end hosts, meaning that viremia production is not high and transmission of JCV to other mammals does not occur (2).

More than 26 species of mosquitoes (9, 17) and 3 species of horse flies (17) transmit Jamestown Canyon virus to people; significant mosquito vectors are from the genera Aedes and Ochlerotatus (9, 17), but Anopheles, Coquillettidia, Culex, and Culiseta species have also been recognized as potential hosts (4, 12, 19). Mosquitoes are also capable of passing the virus to their offspring through vertical (transovarian) transmission (9, 15), which does not occur with other arboviruses such as West Nile and Zika (15). Human infections are most common in northeastern and midwestern states, with the most cases reported in Wisconsin (7, 12). However, the mosquito species that carry the virus have been found in a number of states throughout the country. In a 20-year study (1997-2017), conducted by the Connecticut Statewide Mosquito/Arbovirus Surveillance Program, JCV was detected in 84 of 91 mosquito trapping stations or 92% of the sites (9). Trapping performed in the summer of 2019 has already yielded mosquitoes carrying JCV in at least two states - Michigan and Connecticut (23, 26). Although no human Jamestown Canyon virus cases have been reported in Indiana, several species of mosquitoes that may act as vectors have been trapped in mosquito pools, including Culex restuans, Aedes triseriatus, Coquillettidia perturbans, Anopheles quadrimaculatus, Aedes vexans, and, most significantly, Anopheles punctipennis (12)(27). Due to the variety of mosquitoes that carry JCV, the transmission period of the virus is longer, with cases being reported from late spring into early fall (13)(9, 19, 24). For example, snowmelt Aedes species emerge in early spring and serve as important JCV vectors (3, 24), whereas several other mosquito species transmit the virus in the summer and early fall (3, 12).

No vaccines exists for many arboviral diseases, including Jamestown Canyon virus infection; consequently, prevention is the most important method to combat the disease (4, 7, 8). Precautionary methods recommended include:

- Avoiding or limiting outdoor activities when mosquitoes are most active (late afternoons, nighttime and early mornings)
- Wearing long, loose-fitting clothing that covers exposed skin when present in areas where mosquitoes are observed
- Using mosquito sprays containing EPA-registered repellents such as DEET, picaridin, IR3535, oil of lemon eucalyptus or para-menthane-diol
- Pre-treating clothing with permethrin-based sprays
- Keeping screens on open doors and windows
- Inspecting yards for items or areas that may pool water in which mosquitoes can breed (i.e., tires, wheelbarrows, ditches, puddles)
- Frequently emptying/replacing standing water in flower pots, pet bowls, birdbaths, kiddie pools, etc.
- Cleaning gutters so water does not collect and stagnate
- Filling tree holes that hold water with dirt or sand
- Covering or drilling holes in trash and recycling containers that are kept in open areas outside
- Keeping pools maintained, chemically treated, and/or covered when not in use
- Stocking ornamental ponds with predatory fish such as goldfish, koi and mosquito fish

Additionally, it is recommended that municipal areas practice vector control and health organizations screen blood donations for Jamestown Canyon virus. When the disease does strike an individual, only supportive care is available as no effective protocol for treatment is in place. This includes managing symptoms such as fever, headache, nausea, and vomiting; administering anti-convulsants for seizures; monitoring ventilation and blood pressure; and tending to secondary bacterial infections. In more critical cases, cerebral edema and intracranial pressure may also need to be reduced.

Although Jamestown Canyon virus has received little recognition over the years, health and medical officials have begun to realize that it maybe an arbovirus of significant concern due its widespread distribution, the variety of mosquito species which may act as vectors, and the possibility of serious neuroinvasive complications in infected humans. Until recently, testing for the virus was limited by location and technique. With increased awareness in medical personnel and improved testing methods in laboratories, JCV is now being recognized as a possible pathogen in human neurological cases that occur in areas and seasons in which mosquitoes are abundant. Consequently, more laboratories are preparing to test for JCV, and public officials are cautioning the public to practice prevention against mosquito transmission until more effective treatment and vaccines are discovered.

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# Indiana Non-Fatal Drug Overdose and Abuse Public Health Surveillance – Laboratory Analysis

## By Mary Hagerman, Chemistry Division Director



The recreational use and abuse of drugs continues to impact Indiana. In response to the threat, the Indiana State Department of Health Laboratory (ISDHL) is creating a drug and substance abuse overdose surveillance system that will provide enhanced toxicology testing of clinical specimens. This monitoring will include the identification of many classes of drugs including amphetamines, anti-depressants, anti-psychotics, benzodiazepines and opioids, including over 100 fentanyl analogs and any suspect compounds causing atypical toxidromes or severe illness in our community. This testing does not include synthetic cannabinoids.

The surveillance system relies upon collaboration with our partners in Emergency Departments and hospital laboratories. ISDH is requesting that clinical specimens (blood/urine) be submitted to the ISDH Laboratory for lenhanced toxicological testing that includes analysis for over 700 drugs of abuse, excluding alcohol. This testing is necessary because clinical specimens are often not obtained or tested from non-fatal drug overdose patients, or the current laboratory panels do not include many of the newer synthetic drugs.

Laboratory results will be used for surveillance only. The results will enhance understanding of the drugs/ substances circulating in Indiana communities and will identify those substances causing clusters and severe illnesses; this will inform treatment and prevention approaches. Surveillance data will be available on the **ISDH Opioid Dashboard** along with emergency department (ED) visits, ED hospitalizations, ED syndromic and overdose mortality data.

ISDH is requesting specimens from hospitals where the primary diagnosis is attributed to the use of one or more of the following:

- Schedule I drugs
- Opioids (including prescription opioids, fentanyl, fentanyl analogs)
- Synthetic, non-prescription drugs
- Prescription drugs
- Drug combinations
- Other substances that appear to be part of a cluster of drug overdoses

ISDH is requesting specimens with only the following information. Residual specimens are acceptable.

- Sex
- Age
- Patient ZIP code
- Number of Narcan/naloxone doses if known

ISDH will not report patient specific results back to the hospital or to law enforcement.

Emergency departments or their on-site laboratories are in the process of being contacted by ISDH to discuss any questions and the best procedures for sending specimens to the ISDH laboratory. ISDH can provide postage-paid shipping containers and urine tubes if needed.

Surveillance for drugs of abuse is expected to continue for at least the next three years. As the project progresses, the aggregated surveillance data will be available to all healthcare providers as information that can be used to help decrease the rate of substance abuse disorder.

We would appreciate your participation in non-fatal overdose public health surveillance. This is a project designed to benefit all Hoosiers as we battle Indiana's drug abuse crisis together.

For more information, please contact Mary Hagerman at the ISDH Chemistry Laboratory, <a href="mailto:mhagerma@isdh.in.gov">mhagerma@isdh.in.gov</a> or 317-921-5553.

### **Detection of Sexually Transmitted Infections**

### By Adam Green, Microbiologist

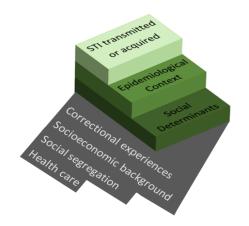


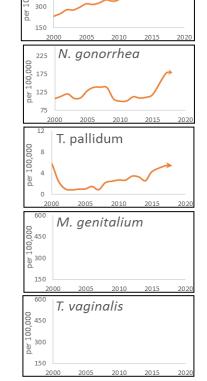
The Indiana State Department of Health Laboratories (ISDHL) provides testing for three sexually transmitted infections (STIs): Chlamydia trachomatis, Neisseria gonorrhea and Treponema pallidum (syphilis). Not every STI has symptoms though and if left untreated, STIs can cause permanent reproductive damage, increased susceptibility to HIV or even death.

There are many factors associated with acquiring STIs: sexual behavior, the number of lifetime sexual partners and social determinants. These factors, mediated by the epidemiological context, can have a strong influence on the |outcome for STIs(1).

Although STIs are on the rise nationwide, we know more about some STIs than others, including Mycoplasma genitalium and Trichomonas vaginalis. M. genitalium is an emerging STI with resistance to antibiotics such as beta-lactams, glyco-peptides, and, in many isolates, azithromycin. T. vaginalis is the most prevalent nonviral STI in the United States, possibly worldwide(2,3).

- M. genitalium symptoms(2)
  - Non-gonococcal urethritis
  - Pelvic inflammatory disease
  - Susceptibility to acquire HIV
  - Spontaneous abortion
  - Pre-term birth
- T. vaginalis symptoms (3) 70% of cases asymptomatic
- Mild-to-severe inflammation
- Susceptibility to acquire HIV
- Low birth weight
- Preterm birth





C. trachomatis

450

We do not have prevalence data for infections with *M. genitalium* and *T. vaginalis* in Indiana residents. We hope to initiate a pilot study to assess the prevalence and burden of *M. genitalium* and *T. vaginalis* infections in Indiana in the near future. Stay tuned for opportunities to participate in this study.

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#### **Got Colistin?**

# By Sara Blosser, PhD, D(ABMM) Clinical Microbiology Division Director



#### **Colistin Testing Drama**

Often considered drugs of last resort, colistin and polymyxin B have been the topic of significant laboratory controversy over the past few years. Many clinicians have wanted to use these drugs for patients with CRE or CP-CRE infections but lacked antimicrobial susceptibility breakpoints.

We do not have AST breakpoints for colistin or polymyxin B. However, in 2018, the Clinical Laboratory Standards Institute (CLSI) published the first guidelines for the interpretation of colistin by way of epidemiological cutoff values (ECVs). But wait! ECVs are not breakpoints, so what's the difference?

#### What are breakpoints?

Dictionary.com says breakpoints are "a place or time at which an interruption or change is made." In the world of AST, a breakpoint is the concentration where decision-making occurs, usually regarding drug choice or dosage. Three main categories of breakpoints are used: susceptible, intermediate, and resistant. Read below to find out a little bit more about what these categorizations mean:

#### Susceptible (S)

Organisms are usually inhibited.

The drug can be dosed at the correct concentration at the site of infection.

The clinical outcome is favorable.

#### Intermediate (I)

Organisms can be inhibited, but not as often as for those that are susceptible. If the drug can reach the site of infection at the right dose, it could be effective.

#### Resistant (R)

It is unlikely that an effective drug dosage can be achieved at the site of infection.

The clinical outcome is not favorable (i.e. choose a different drug).

#### How are breakpoints established?

Have you ever considered what data is needed to establish AST breakpoints? It's more complex than you may think! Three major components go into establishing a breakpoint.

First, the normal or wild-type distribution of MICs for an organism against a particular drug must be considered. In the wild, organisms will naturally gravitate toward a particular minimum inhibitory concentration (MIC) for each drug. This is called the wild-type (WT) distribution. Isolates that exhibit MICs higher than the wild-type distribution tend to be those that have developed or acquired resistance. These isolates are referred to as Non Wild-Type (NWT). Being WT is not the same as being susceptible, however, as isolates that are intrinsically resistant to a drug have a WT distribution.

Second, the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug need to be considered. PK and PD evaluate essential items, such as the drug's effectiveness over time, the ability of the drug to reach the area of disease, or the metabolism and excretion characteristics of the drug in a patient's body. Here's an example from ceftazidime and the Enterobacteriaceae: when ceftazidime is dosed 1g every 8 hours, target rates are reached >90% of the time when an isolate has an MIC of 4  $\mu$ g/mL, but only 40% of the time if the isolate has an MIC of 8  $\mu$ g/mL. It makes sense, then, that the susceptible MIC for ceftazidime for the Enterobacteriaceae is  $\leq$  4  $\mu$ g/mL, not 8  $\mu$ g/mL.

Third, clinical outcome trials must be examined to evaluate whether what works in theory actually works in practice. A great example of this was a 2018 study that evaluated the effectiveness of piperacillin-tazobactam (pip-tazo) compared to meropenem for the treatment of ceftriaxone-resistant *E. coli* or *Klebsiella pneumoniae* bloodstream

infectious. Logic tells us that if an isolate is susceptible to both pip-tazo and meropenem, that it is better from an antibiotic stewardship perspective to use pip-tazo. Based on the study, however, pip-tazo was shown to be inferior to meropenem at 30 days (all-cause mortality, 12.3 vs. 3.7%). My conclusion? When it comes time to review the pip-tazo breakpoints, the folks at CLSI will evaluate these types of studies to ensure that their recommendations are of sound scientific judgment and reflect best practices.

Once all three pieces of data are combined, the breakpoint is established by a panel of experts, and published in the most recent CLSI-M100 document.

#### So what are ECVs?

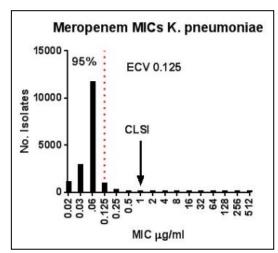
ECVs, on the other hand, only consider the WT-distribution of the organism. Specifically, an ECV is established where ≥ 95% of the wild-type population MICs 'sit.' CLSI typically uses this designation when there is not enough PK-PD or clinical trial data to draft breakpoints for a particular drug-bug combination. Colistin is a prime example!

Not convinced? Here's an example for K. pneumoniae and meropenem – an example where both an ECV and true breakpoints have been established. The ECV for K. pneumoniae with meropenem is  $0.125 \, \mu g/mL$ . This is where  $\geq 95\%$  of wild-type K. pneumoniae will 'sit'. The susceptible breakpoint, however, is  $\leq 1.0 \, \mu g/mL$  because PK-PD and clinical studies were also evaluated.

#### So what do I report to my clinician?

The funky thing about ECVs is the way they are reported. Instead of reporting S/I/R, drugs like colistin should be reported as WT or NWT. Everything below the ECV is WT, everything above the ECV is reported as NWT.

Another thing to know is that we can't report ECVs for all Enterobacteriaceae, only *Klebsiella aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae* and *Raoultella ornithinolytica*. ECVs have to be determined species-by-species because of the significant genetic diversity between different species within the same genus or family.



#### What's the take-home message?

We now have interpretations for colistin testing, but they're far from ideal. In general, clinicians are more comfortable with a result of S/I/R than they are of WT/NWT. So how are they used? In general, if an isolate is NWT for a particular drug, that drug should not be used. If an isolate is WT for a particular drug, however, that drug may be effective. In short, it gives the clinician a rough guideline for drug usage but requires them to consult with peers or their own consensus bodies, such as the Infectious Disease Society of America (IDSA) to make these tough decisions.

Want to know more? Check out this cool website (<a href="http://www.uphs.upenn.edu/bugdrug/antibiotic\_manual/ECV.htm">http://www.uphs.upenn.edu/bugdrug/antibiotic\_manual/ECV.htm</a>).

# What's in your Water? Part Deux By Mary Robinson, Water Microbiology Laboratory Supervisor





Credit: https://www.epa.gov/water-research/epidemiology-health-effects-cyanobacteria

At ISDHL, our most common water-related questions are concerns regarding standard bacterial and chemical contaminants, such as total coliform bacteria, heavy metals, etc. But callers who drink out of reservoirs and bathing beach goers face another concern, "blue-green algae" cyanobacterial toxins.

Cyanobacteria are photosynthetic and aquatic bacteria often called "blue-green algae" (BGA); they grow in shallow, slow moving or still water. Their colors can range from olive-green to red. When the amount of BGA in the water increases, a large dense mass may form called a bloom. Blooms cover the surface of the water and can look like thick pea soup. They are often blue-green in color and may produce toxins. Blooms are often referred to as "harmful algal blooms" or "HABS". Not all blooms are easy to see; toxins can still be in the water even if you cannot see the blooms. BGA blooms occur naturally and are often found in water enriched with nutrients from human activities such

as municipal, industrial or agricultural sources. The presence of BGA toxins at public water system (PWS) intakes along Lake Erie and the Ohio River have made news headlines.



Credit: https://www.cdc.gov/habs/index.html

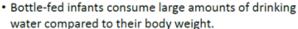


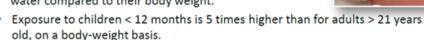
Some BGA blooms can produce chemicals often referred to as cyanotoxins which are poisonous if swallowed by people, pets, or livestock. As with many water contaminants, the human population most vulnerable based on drinking water consumption are children.

There are several types of toxins that can be produced by blue-green algae. Cyanotoxins are often categorized into two groups based in their physiological effects: 1) Neurotoxins affect the nervous and respiratory systems and can cause muscle tremors, stupor, staggering, rapid paralysis, breathing problems and in extreme cases, death. Pets and livestock that die from these toxins are usually found close to the body of water where they drank. 2) Hepatotoxins affect the liver and can take days before symptoms appear after drinking affected water. Symptoms often include jaundice (yellowing of the white of the eye) and sensitivity to sunlight.

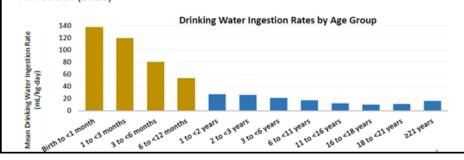
Typical BGA testing involves; 1) identification and quantification of species to monitor for an increase in overall BGA population and an increase in specific species known to produce toxins, and 2) quantification of toxin through immunological or analytical methods such as ELISA, LC/MS/MS, LC-UV, etc. Toxin compounds are biosynthetically-

## Children's Exposure to Cyanotoxins





- At 6 years and older, exposure on a body-weight basis is similar to that of an adult.
- Infant-specific exposure factors are available from U.S. EPA's Exposure Factors Handbook (2011)



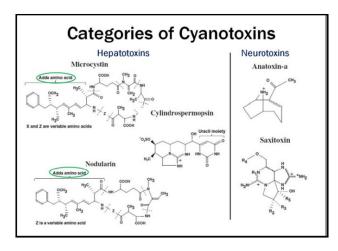
 $SOURCE: \\ \underline{https://www.epa.gov/sites/production/files/2015-07/documents/webinar-health-advisories-cyanotoxins-2015.pdf}$ 

produced secondary metabolites; there is little understanding as to why and specifically, when they may be produced. In addition, each toxin may have several variants/ congeners (additional constituents), creating detection and identification even harder.

To be proactive, it is desirable to perform testing to detect the presence/increase in BGA and cyanotoxins before toxins are produced/released. The ISDH Water Microbiology Laboratory is under the guidance of the Ohio Environmental Protection Agency Division of Environmental Services Harmful Algal Blooms, Microbiology, and Certification Division (Ohio EPA) and is financially supported by the Indiana Department of Environmental Management Office of Water Quality

(IDEM OWQ). The laboratory began Real Time qPCR implementation for the detection and quantification of cyanobacterial 16S and toxin genes in PWS water at high risk for cyanobacterial toxin production, utilizing the Phytoxigene CyanDTec multiplex quantitative multiplex PCR (qPCR) assay method. The Ohio EPA, IDEM OWQ and others actively collect and analyze data on genes, detected toxin, and other key environmental factors to understand more about what causes BGA growth and toxin production.

This genetic test approach offers a broad level of sensitivity and information independent of cell characterization and has the ability to assess homogeneity/heterogeneity of cyanobacterial populations, as not all cells are toxin-producers and not all blooms are individual species driven. This offers the potential for earlier intervention and the ability to discriminate or validate follow-up tests, as possible congeners or species may not be identified as being toxin producers. This test also measures the toxin pathway gene number independent of cell count and monitors toxin and non-toxin cyanobacteria population dynamics. Genetic testing makes it feasible to monitor and test more frequently, can be predictive and more sensitive than current morphological identification methods, is non species-dependent, provides rapid turnaround of results, and reduces need/frequency of higher cost taxonomic identification and analytical methods.

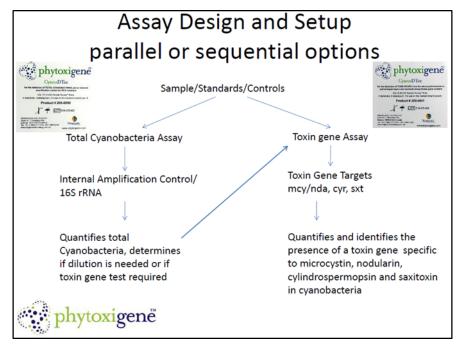


Phytoxigene™ CyanoDTec is the first standardized cyanobacterial toxin gene assay. The assay detects and quantifies the presence of Cyanobacteria and their toxin-producing genes from aquatic environmental samples. The test quantitates both the amount of overall Cyanobacteria gene copies present in a sample, along with the number of genes that are responsible for the production of the toxins. Both hepatotoxins and neurotoxins are detected with this test; the hepatotoxins include microcystin, nodularin and cylindrospermopsin, while saxitoxin is one of the primary neurotoxins produced by Cyanobacteria. The method simultaneously identifies and quantifies the presence of total cyanobacteria along with three genes responsible for four different toxins' production; total Cyanobacteria (16S rRNA gene), Microcystin and Nodularin (mcyE/ndaF gene), Cylindrospermopsin (cyrA gene) and Saxitoxin (sxtA gene).

(continued on next page)

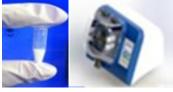
## What's in your water? (continued from page 9)

The CyanoDTec assay design is as follows:



The assay uses extracted DNA obtained from water sample filtration, followed by the mechanical lysing of the cells. Lysed cells are centrifuged, and the supernatant is extracted as the sample PCR template. Assay steps in brief:

- Vigorously shake samples and perform membrane filtration.
- Lyse cells by placing filter into the bead lysis tube and homogenize.
- Centrifuge bead lysis tube; extract supernatant for PCR analyses.
- Re-hydrolyze master mix.
- Add 5 μl of sample to 20 μl of master mix in 96-well microtiter plate well; gently agitate, then centrifuge plate.





rsis centrifuge t

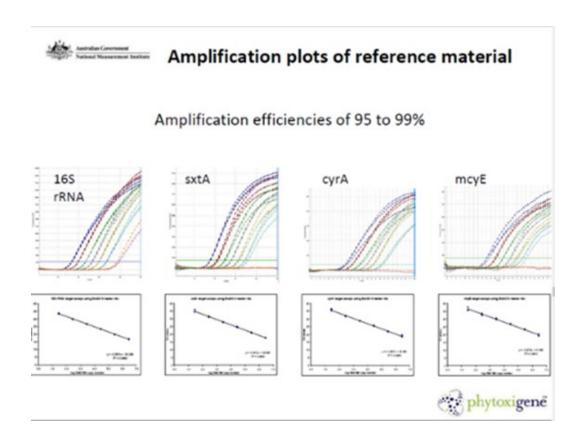
thermocycler

- Select the following cycling conditions on thermocycler: 2 minute initial denaturation at 95°C; 15 second denaturation at 95°C; 30 second annealing/extension phase at 60°C. Set number of cycles to 40. Data collection takes place during the annealing/extension phase.
- Place in thermocycler and initiate run; total time 2-3 hours.

The assay includes a set of six known gene copy concentration standards used to create a standard curve. The sample gene copy results are based on the sample Ct (cycle threshold) values compared to known concentration standard curve Ct values.



| Standard (cap color) | Copies/ul         | Copies per reaction       |
|----------------------|-------------------|---------------------------|
| NA011 (white)        | 0 copies/ul       | 0 copies (BLANK)          |
| NA012 (green)        | 20 copies/ul      | 100 copies/reaction       |
| NA013 (yellow)       | 200 copies/ul     | 1,000 copies/reaction     |
| NA014 (blue)         | 2,000 copies/ul   | 10,000 copies/reaction    |
| NA015 (orange)       | 20,000 copies/ul  | 100,000 copies/reaction   |
| NA026 (pink)         | 200,000 copies/ul | 1,000,000 copies/reaction |



The results provide information on: the presence of Cyanobacteria, an indicative measure/level of Cyanobacteria; the presence of one or more toxin genes; the amount of toxin gene; and information on the percent gene load to total cyanobacterial content . The goal of ISDHL is to provide IDEM a quick turn-around time for cyanobacterial 16S and toxin Gene Copy/µl test results. The data will be used to make correlations between drinking water system monitoring data and become a predictive assay before a cyanotoxin release event occurs.

#### For further reading:

https://www.epa.gov/cyanohabs

https://www.in.gov/idem/algae/

https://epa.ohio.gov/ddagw/HAB

https://www.phytoxigene.com/resources

# Insuring Healthcare and Lab Safety One Swab at a Time By Mark Glazier, Biological Preparedness, Laboratory Outreach and Logistics Division Director



In laboratory science, things that might appear to be inconsequential often have a significant impact on the final testing result. A good example of this is the collection of a quality patient specimen. Jyl Madlem, the ISDH Laboratory program advisor, emphasizes the importance of quality blood specimen collection during her phlebotomy refresher trainings for nursing personnel. During these trainings, she also demonstrates proper collection of a nasopharyngeal (NP) swab specimen. Demonstration of proper NP swab collection was added to the phlebotomy training after the H1N1 influenza outbreak because the lab received a large number of nasal swab specimens instead of the preferred NP swab specimen.

Over the years of providing this training, Jyl noticed a couple of issues with the design of the Puritan NP swab used for the collection demonstration. Jyl thought it would be good if the company knew about the issues, but she never believed the opportunity would play out as it did.

I had the opportunity to get more specifics from Jyl regarding her observations.

# MG: Jyl, tell me about how you were able to bring the swab issues to Puritan Medical Products Company's attention?

JM: I was at the American Society for Microbiology annual meeting in New Orleans in June 2017, perusing the vendor exhibits, and I decided to stop by the Puritan booth. I was asked by Michael Keyes, vice president of microbiology sales, what I thought about their NP swab collection kit. I explained that the current swab design had the potential to scratch the inside of the nasal cavity. I also let him know another potential problem with the swab was the scored break point of the swab. When the swab is put into the viral transport media, the score line is above the top of the tube, creating a potential splash hazard for the collector, as well as the receiving laboratory.

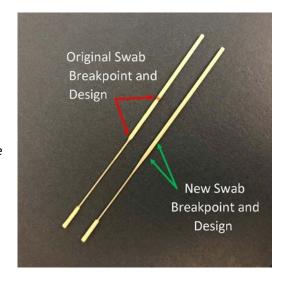
#### MG: What did Puritan think about your assessment of the swab?

JM: It turned out that several senior members of the Puritan Medical Products team, including the president of the company, were interested in my assessment.

#### MG: What happened next?

JM: In August 2017, Puritan sent me a couple mock up redesigns of the swab that they coined the 'Jyl Swab.' According to Michael Keyes, the challenge was developing a collection swab with the current Puritan Medical Products "Ultra flocked tip" and a shaft redesign to provide more patient comfort and reduce the "springing" effect of the current shaft design when placing the swab into the transport media vial for viral transport and eventual testing. I tried out the redesigned swab at several of my phlebotomy trainings.

MG: Did that end up being the final design of the new swab? JM: Yes, the new swab design addressed both issues noted above.



#### MG: What are the next steps for the 'Jyl Swab Project'?

JM: Manufacturing of 600-900 devices is to be completed and sent to the ISDH Laboratories by late August to be used for the upcoming Indiana influenza sentinel site surveillance program as part of an evaluation and validation study. Puritan will also be creating a short questionnaire to be sent with the swab collection kits to assess how sentinel sites like the new swab design.

#### MG: Is there anything else you'd like to add about this endeavor?

JM: I was shocked that they listened to my opinion. Don't be afraid to let others know your opinion because it just might end up having a much larger impact than expected.

# Employee Spotlight: Marissa Kozlowski By Jamie Yeadon-Fagbohun, Enteric/Parasitology Laboratory Supervisor





Marissa Kozlowski is a microbiologist in the enteric, parasitology and molecular subtyping section of the laboratory. She started at ISDHL as a contractor in February of this year. Marissa is originally from Northwest Indiana near Chicago. She graduated from IUPUI in 2018 with a bachelor's degree in biology.

Marissa's main responsibility in the laboratory is to prep isolates and extract the DNA so they are ready for whole genome sequencing. She also assists with the receiving and plating of specimens and serotyping of isolates as time allows. As she continues her training within the laboratory section she will eventually learn all of the assays completed by the Enteric laboratory.

So far some of her position have been learning new techniques and being part of the Salmonella serotype Carrau outbreak in April of this year. Marissa stated "It was a great learning experience and very fulfilling to work with such a great team." As for future plans, Marissa plans to continue cross-training and learning new techniques and expanding her knowledge. She is also considering returning to school to further her education.

Outside of work, Marissa enjoys spending time with animals awaiting adoption at the local animal shelters. She lives with her fiancé in downtown Indianapolis with their two cats, Luna and RuRu. Marissa is busy planning her wedding for the fall 2020. She is excited for the new opportunities at ISDHL and enjoys the work environment. Welcome, Marissa!

# **About The LAByrinth**



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